Effects of Beta-carotene Administration on Fertility in Lactating Dairy Cows

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ABSTRACT

Background: Beta-(β-) carotene, is the precursor to vitamin A, in particular, has some potential benefits on reproduction. The main objective of this study was to investigate the efficacy of β-carotene administration on fertility following either prostaglandin F2 alpha (PGF) induced estrus or Ovsynch protocol in lactating dairy cows.

Methods: Cows with at 47±3 postpartum days were divided into two groups: β-carotene group (βC, n=139) was treated with injectable β-carotene while untreated cows served as control (CON, n=227). In both groups, PGF was administered and heatmount detectors were applied at 54±3 days postpartum. Cows detected in estrus after PGF were inseminated. Cows that had not been detected in estrus were divided into two groups 7 days after PGF administration; βC-OVS (n=137) and CON-OVS (n=89). Ovsynch protocol was initiated 4 days after β-carotene administration.

Result: The estrus detection rate was similar between the βC and CON groups (P = 0.19). Pregnancy per AI (P/AI) on d 31 was also similar between groups (P = 0.93). In the Ovsynch protocol, ovulation to the first GnRH and ovulatory follicle diameter at the time of insemination did not differ between groups. No difference was observed in P/AI at d 31 (P = 0.13). The results of this study indicated that β-carotene administration had no effect on fertility either PGF induced estrus or Ovsynch protocol in dairy cows.

Key words: βeta-carotene, Estrus detection, Lactating dairy cows, Synchronization.

INTRODUCTION

Detection of estrus is considered as one of the most important factors affecting fertility in dairy cows. However, the rate of estrus detection is below 50% in many commercial dairy farms (Roelofs and van Erp-van der Kooij, 2015; Unalan, 2016). Prostaglandin F2α (PGF) and its analogues have been used to manipulate the bovine estrous cycle successively. Also, timed artificial insemination (TAI) programs have become important tools for reproductive management which allowing insemination without the detection of estrus signs (Pursley *et al*. 1997). Ovsynch is the most used TAI program to control the first and subsequent AI's with sufficient pregnancy rates.

It is known that a suitable concentration of beta-carotene is closely associated with bovine health and fertility. It has been postulated that low circulating βC has been associated with decrease the intensity of the signs of estrus, delayed ovulation, low luteal function and low conception rate (Graves-Hoagland *et al*. 1989; Sales *et al*. 2008; Ay *et al*. 2012; Madureira *et al*. 2020). On the other hand, this has been a controversial topic; some researchers have reported no effect of βC on reproductive performance (Bindas *et al*. 1984; Jukola *et al*. 1996). These variations observed among the studies might be depending on the amount and duration of βC supplementation, breed, postpartum stage, environment and management.

The objectives of this field study were; 1) to determine whether a single injectable βC administered before PGF could increase expression of estrus and pregnancy rate, 2) to evaluate whether administration of βC before Ovsynch would affect ovulatory response to the first GnRH, synchronization rate, ovulatory diameter at AI and pregnancy rates in lactating dairy cows. Thus, the aim was to investigate the effects of injectable βC on reproductive performance at different situations whether with induced estrus or TAI protocol in lactating dairy cows during the early postpartum period.

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MATERIALS AND METHODS

Animals

A total of 366 Holstein Friesian cows without any postpartum problems, such as retained fetal membranes and acute metritis, were enrolled in the study. A well-managed, high-producing commercial dairy herd located in the South Marmara region of Turkey was used. Cows were kept in free-stall facilities and during the hotter months, barns were cooling with sprinklers and fans. According to milk production, cows were grouped and were fed with a total mixed ration compatible following the National Research

Council (National Research Council, 2001) recommendations. The ALPRO®2000 system was used to collect management records for each cow. The mean milk production over 305 days of the herd was 9.880 kg/per cow. Body condition scores (BCS) were determined with a scale from 1 to 5 (1= very thin to 5 = obese) (Ferguson *et al*. 1994). Animal procedures were approved by Lalahan Livestock Central Research Institute Animal Care Committe.

Study design

The cows (n=366) were randomly assigned to the treatment and control group at 44 to 50 days in milk (DIM); β-carotene treatment group (βC, n=139) and control group (CON, n=227) (Fig 1). Cows in the βC group received an injection of βC (200 mg/cow, im, Carofertin®) which dose was determined following the manufacture's recommendation. The total dose of βC was divided into two doses and injected in different areas of the rump. At 51-57 DIM, ovarian structures were determined by ultrasound (Honda HS 2000®) equipped with a 7.5 MHz linear-array transrectal transducer. All cows in treatment and control groups were injected with PGF2α (5 mL/cow, im, Enzaprost®), heatmount detectors (Kamar®) was applied concurrently with PGF injection and was checked three times for seven days to detect estrus. Cows were inseminated after detected in estrus.

At 58 to 64 DIM, cows not detected in estrus were enrolled in two groups; β-carotene injection (βC-OVS; n=137) was applied 4 days before initiation of Ovsynch cows in the βC-OVS group and untreated cows were used as a control (CON-OVS; n=89) (Fig 1). The Ovsynch protocol was applied all cows in both groups. First GnRH (2.5 mL/cow, im, Oviren®) of Ovsynch was administrated; PGF treatment was performed 7 days later; a second GnRH was injected 56 h later. At 72 to 78 DIM, fixed TAI was applied all cows 18 h after the second GnRH injection. At the time of the first GnRH treatment in Ovsynch, ovaries were scanned to obtain the CL and follicles. Ovaries were checked 7 days later to determine response to the first GnRH treatment. Maximal follicle diameter was measured for each follicle before the TAI. Disappearing of the previously measured ovulatory follicle and determining a new CL at 7 days after TAI was accepted the ovulatory response to the second GnRH. Pregnancy was diagnosed on 31 d and presence of a fetus on 62 d.

Statistical analysis

Statistical analysis were performed using SAS® (Version 9.4, SAS Institute)[7]. Data were evaluated using PROC LOGISTIC, PROC GLM and PROC FREQ in SAS. Before data analyses, continuous outcomes were tested for normal distribution using Shapiro-Wilk's method. A GLM procedure was performed to compare the means of milk production, BCS, parity and follicle sizes between groups. Chi-square analysis using the PROC FREQ procedure was used for testing the association between the presence of CL at the time of PGF and beginning of the Ovsynch, estrus detection rate, response to first and second GnRH of Ovsynch and P/AI (31 and 62 d). A multiple logistic regression model was conducted to compare the effects of milk production, BCS, follicle size at the time of AI, parity, CL presence at the time of PGF and beginning of Ovsynch, response to first and second GnRH of Ovsynch and treatments on conception rates at 31 and 62 d. For the multiple logistic regression model, milk yield, parity, BCS, follicle size at the time of AI, treatment groups, cyclicity before Ovsynch and response to first and second GnRH of Ovsynch were considered as explanatory variables and P/AI at 31 and 62 d were considered as outcome variables. A stepwise selection procedure was utilized to construct the final model. A level of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The authors suggested that βC may positively effect on uterine involution, ovulation rates, progesterone concentrations, conception rates and may reduce the incidence of weak estrus expression, cystic ovaries, early embryonic loss (Kaewlamun *et al*., 2011; Ay *et al*., 2012; De Gouvêa *et al*., 2018; Madureira *et al*. 2020). Previous studies reported that an increase in blood serum βC concentrations determined following parenteral administration of βC (Emre *et al*., 2018) and feed supplementation (Oliveira *et al*., 2015). Unfortunately, as a limitation of this field study, we did not collect or analyze blood samples after administration of βC, however, studies were reported that the blood βC concentration significantly higher one week after parenteral administration (Emre *et al*., 2018; Hye *et al*., 2020).

Detection of estrus is the first step for the successful management of reproduction in dairy farming. Milk yield, lameness, poor nutrition and herd size are some factors

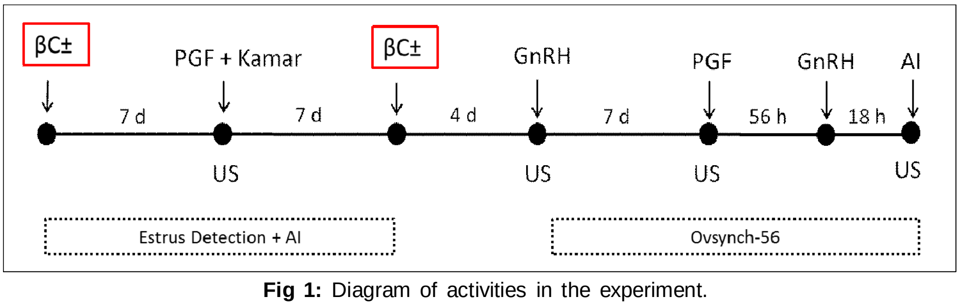

Fig 1: Diagram of activities in the experiment.

βC = Injection of 200 mg of beta-carotene; GnRH = Injection of 10 µg of buserelin acetate; PGF = Injection of 25 mg of dinoprost as tromethamine; US = Ultrasonography; AI = Artificial insemination.

Table 1: Mean lactation number, milk production and BCS in β-carotene (βC) and control (CON) groups before PGF administration and Ovsynch protocol.

Item	βC	CON	P
PGF protocol			
Mean lactation number	2.63±0.38	2.68±0.30	0.59
Milk production (kg/d)	41.39±0.60	40.15±0.50	0.12
BCS	2.63±0.38	2.68±0.30	0.45
Ovsynch protocol			
Mean lactation number	2.62±0.11	2.49±0.16	0.12
Milk production (kg/d)	42.23±0.62	40.24±0.75	0.04
BCS	2.63±0.03	2.67±0.04	0.50

that can affect the performance of estrus detection. Also, PGF is widely used for the induction of estrus in many farms; artificial insemination is performed after the expression of estrus. The mean lactation number, milk production and BCS for cows were similar between the groups before induced estrus with PGF administration. In addition, lactation number and BCS did not differ between the group at the regrouping before Ovsynch protocol, however, average milk production was found to differ (P=0.04) between groups (Table 1).

The percentage of cows with CL at the time of PGF injection were determined by 73.6% (254/345). As shown in Table 2, the rate of cows with a CL was found to be similar (P=0.33) between groups. After the PGF, 119 cows were determined to be in estrus and they were inseminated. Estrus detection rate was similar between groups (P=0.23), similarly to the observations of others (Akordor *et al.*, 1986; Oliveira *et al*, 2015). Pregnancy per AI at 31 d was not different (P=0.93) between βC (42.5%) and CON (41.7%) groups and 62 d was also similar. Besides, according to logistic regression, regardless of the treatment, when evaluating the effect of BCS (P=0.48), CL at the time of PGF (P=0.52), milk production (P=0.40) and parity (P=0.96) did not affect P/AI. In contrast, Ay *et al*. (2012) observed that the administration of βC especially applied during the postpartum d 15 and 45 improved PGF induced sexual cycle's synchronization and fertility parameters. It has also been reported that even a single injection of βC during the postpartum period before PGF injection enhances fertility parameters (Ay *et al*, 2012). Madureira *et al*. (2020) reported that even though cows were fed the same diet, the blood concentration of βC determined differently from each cow which can be related to individual animal factors of βC absorption, milk production and BCS (Ashes *et al*, 1984; Weiss 1998). In addition, the authors declared that cows with a greater concentration of βC in blood during the TAI had better P/AI. In the present study cows in the βC and control groups had similar lactating numbers, milk production and BCS (Table 1) so we assumed that based on previous studies, administration of βC would increase its concentration in the blood, which would have a positive effect on estrus detection and pregnancy rate. However, we failed to identify a positive effect of βC administration either on the estrus expression or the pregnancy rate after the induction estrus with PGF. The reason for these contrasting results may be, the increased blood level of βC concentration after one application might not be sufficient to detect a positive impact on the fertility parameters. On the other hand, a correlation between the sustained higher level of βC concentration in blood and improved fertility parameters was found (Aréchiga *et al.*, 1998).

Synchronization protocol such as the Ovsynch protocol allows for the synchronization of follicular development, luteal regression and timed artificial insemination (Pursley *et al.*, 1997). The success of the Ovsynch program depends on the response to each hormone administration during the protocol. Besides, the cyclicity status of cows at the start of Ovsynch affects the success of the protocol (Martins and Pursley, 2016). As shown in Table 3, the rate of cows with a CL at the beginning of Ovsynch was similar among the groups. The ovulatory response to the first GnRH and synchronization rate were not affected by the administration of βC in the present study. The results of ovarian activity observed in this study were in agreement with those reported by Celik *et al.* (2009) who conducted a study with repeat breeder cows and showed that ovulation response did not differ between control and βC groups after first and second GnRH injection. However, only repeat breeder and a small number of cows were used in the study (Celik *et al.* 2009). Moreover, there is no study to compare the results of the synchronization response in early postpartum lactating cows

Table 2: The effect of β-carotene after PGF induced estrus on fertility.

Group	EDR before PGF (%)	CL at time of PGF (%)	EDR after PGF (%)	P/AIat 31 d (%)	P/AIat 62 d (%)
βC	5.0(7/139)	76.5 (101/132)*	30.0(40/132)	42.5(17/40)	42.5(17/40)
CON	6.2(14/227)	71.8(153/213)*	37.1(79/213)	41.7(33/79)	41.7(33/79)
P	0,65	0,33	0,19	0,93	0,93

EDR: estrus detection rate. *14 cows in CON and 7 cows in βC excluded due to inseminated before PGF.

Table 3: The effect of β-carotene administration on Ovsynch outcomes.

Groups	Cows with CL at the beginning of Ovsynch (%)	Response to first GnRH of Ovsynch (%)	Response to second GnRH of Ovsynch (%)	P/AI at 31 d (%)	P/AI at 62 d (%)
βC-OVS	71.5(98/137)	78.8(108/137)	90.5(124/137)	37.2(51/137)	35.0(48/137)
CON-OVS	67.4(60/89)	73.0(65/89)	84.7(76/89)	47.2(42/89)	42.7(38/89)
P value	0,50	0,31	0,25	0,13	0,24

as obtained from this study. Ovulatory follicular size at the time of AI is a critical factor affecting the efficiency of Ovsynch. Except for the findings of De Bie *et al*. (2016) who described follicular diameter increased in positive energy balance cows after supplementation with βC, ovulatory follicle diameter was not affected in our results. Besides, Celik *et al.* (2009) reported that the size of preovulatory follicles in the βC group was more narrowly distributed than control follicles.

It has been suggested that βC was shown to have a positive effect on luteal progesterone (Celik *et al.* 2009; Green and Fascetti, 2016), while others have not reported (Kaewlamun *et al.*, 2011; Ay *et al.*, 2012). As well known, this is an important factor in the improvement of reproduction and reduced early embryonic mortality. Besides the similarity in synchronization responses to Ovsynch in both groups, no statistically significant effect was detectable of the administration of βC on pregnancy rate the same as the study of others (Kacar *et al.*, 2008; Celik *et al.* 2009). Results from Ay *et al.* (2012) also suggest that the injection time of βC is important to find beneficial effects on fertility. But, in our results, we could not obtain the positive effect of βC administration on fertility even though βC injection applied on d 47±3 postpartum before induced estrus and on day 61±3 postpartum for Ovsynch program.

CONCLUSION

In conclusion, the administration of βC before PGF induced estrus or Ovsynch synchronization protocol did not improve estrus signs, synchronization efficiency and P/AI in early lactating dairy cows. This field study suggests that keep the level of βC in the blood higher for a longer period of time might be better than one or two administrations and the deficiencies or conditions in the farm should be better identified. Further studies are necessary to investigate the intriguing physiology relationship between βC and fertility.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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